

COMPARISON OF TWO MORPHOMETRICS METHODS FOR DISCRIMINATING OF HONEY BEE (*APIS MELLIFERA MEDA* SK.) POPULATIONS IN IRAN

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ABSTRACT

In this study, we compared two different methodologies (standard morphometrics and geometric morphometrics) in order to determine their ability to discriminate the honey bee populations distributed in Iran. In standard morphometrics, four distances and eleven angles were measured from the forewings of different honey bee populations. A total of 18 landmarks were utilized for the geometric morphometrics analysis. All colonies, described by mean of 40 workers, were correctly classified using both methods. In the case of individual wings discrimination, geometric morphometrics was 83.83% successful and standard morphometrics was 52.77% successful while the geometric morphometrics method is much simpler and easier compared to the standard morphometrics. Therefore, the discrimination ability of geometric morphometrics on the honey bee populations was better than standard morphometrics.

KEYWORDS: Geometric Morphometrics, Standard Morphometrics, *Apis Mellifera Meda*, Iran

INTRODUCTION

Two hypotheses have been stated about evolution of honeybees. According to the one hypothesis, honeybees evolved in near Ethiopia in Africa and extend over Africa and then Middle East and Europe (Wilson 1971). However the second hypothesis explains that, although representatives of most of bee species were original to all the continents, bees belonging to the genus *Apis* were originally to be found only in the Old World, namely southern part of Caspian Sea and they were distributed to Africa and Europe (Ruttner 1988). According to Ruttner (1988), there are at least 24 *Apis mellifera* subspecies grouped in three or four evolutionary branches, based on morphometric data. Ruttner *et al.* (1985) discriminated six local populations for *A. m. meda*, as follows: West and Central Iran (Azerbaijan-Iranian highlands), the subtropical coast of the Caspian Sea (Mazandaran), Northeast Iran (Mashad), Southeast Iran (Kerman), Iraq, and Southeast Anatolia, from Van Lake to Hatay.

Morphometrics has been used widely for identifying honey bee races, because of high practicability and low costs. The first effective procedure, developed in the 1970s, consisted of manually measuring 25 characters of the wings, sternites and legs with an ocular micrometer; these measures were then analyzed with multivariate statistics to make the identifications (Daly and Baling, 1978).

The field of geometric morphometrics is relatively new (see Bookstein, 1991, Rohlf and Marcus, 1993) and has shown very rapid progress over the last few years. As might be expected during a period of rapid development, there can

be technical problems in some of the pioneering studies as biologists attempt to apply the new tools. It is only now becoming clear how the new techniques should be combined in order to carry out comprehensive analyses of real datasets. Bookstein (1996b) gave a list of recommendations for such applications and Bookstein (1996a) gave several comprehensive examples of morphometric analyses. However, these accounts do not address some of the types of applications that are of particular interest in systematic biology. Bookstein (1994) emphasized that morphometrics cannot supply homologous shape characters.

The aim of this study was comparison of effectiveness of geometric morphometrics and standard morphometrics as methods for discriminating honeybee populations based on forewing venation.

MATERIALS AND METHODS

A total of 10 colonies were sampled from 10 locations belonging to different geographic regions in Iran (Sanandaj: 35 19' N; 46 6' E, Dehgolan: 35 16' N; 47 25' E, Qorve: 35 10' N; 47 48' E, Marivan: 35 31' N; 46 10' E, Bane: 35 59' N; 45 53' E, Saqqes: 36 14' N; 46 16' E, Divandare: 35 54' N; 47 01' E, Sarvabad: 35 18' N; 46 22' E, Bijar: 35 52' N; 47 36' E, Kamyaran: 34 47' N; 46 56') table 1. Honey bees were preserved in preservative solution (30 parts distilled water, 15 parts 95% ethanol, 7 parts 38% to 40% formaldehyde and 2 parts acetic acid) to prevent sample deformation. The right forewings of 40 worker honeybees per colony were mounted by microscope slides. Digital photos were taken from mounted wings using a DP12 camera and SZX12 OLYMPUS stereomicroscope.

Standard Morphometric Analysis

The total of 14 morphometric characters were biometrically measured with the tpsdig 2.16 (Rohlf, 2010). Morphometric characters included forewing length (FL), forewing width (FB), cubital index ($\frac{C}{B}$) (Figure 1), and eleven forewing angles (A4, B4, D7, E9, G18, J10, J16, K19, L13, N23, O26) (Figure 1). The mean values of measurements of 40 honey bees in each colony were calculated. To discriminate the honey bee populations based on morphometric characters, multivariate statistical analyses were performed on measurement values. Statistical analyses were carried out with the computer software programs SPSS 17.0 and NtSys Pc. 2.

Geometric Morphometric Analysis

Eighteen landmarks on fore wings were digitized on photos by tpsDig 2.16 software (Figure 1). The vein junctions were used as homologous points for geometric morphometrics (Rohlf, 2010). Later, landmarks were superimposed using a generalized procrustes analysis (GPA; Rohlf, 1999). All non-shape variations of these landmarks such as orientation (or rotation), scale, and size were removed. Next, digitized landmark outputs were analyzed by tpsRelw, tpsReg (Rohlf, 2010), and NtSys Pc. 2. Finally, samples of ten areas were classified with discriminant function analysis (DFA) by SPSS ver. 18. A multivariate analysis of variance (MANOVA) was carried out on the landmark data in order to compare honey bee populations.

RESULTS

Analysis of variance (ANOVA) of morphological characters (standard morphometrics) showed that 12 out of 14 characters (i.e. all except E9 and L13) displayed statistically significant differences among honey bee populations ($P < 0.05$). E9 ($P = 0.117$) and L13 ($P = 0.08$) characters showed no significant differences between populations. Analysis of

variance (ANOVA) of morphological characters (geometric morphometrics) showed that all variable (1x, 1y to 16y) displayed statistically significant differences among honey bee populations ($P < 0.05$).

All colonies were classified correctly using both the classification functions based on standard morphometrics (Appendix I). A multivariate analysis of variance (MANOVA) displayed statistically significant differences among honey bee populations in standard morphometrics ($F = 6.74$, $P = 0.00$) table 2 and geometric morphometrics ($F = 8.03$, $P = 0.00$) table 3.

Populations of ten areas based on 32 shape variables of fore wings were classified by Discriminate Analysis (DFA) in geometric morphometrics. Cross-validation tests based on DFA correctly classified 83.83% of the colonies table 4. DFA showed that honey bee population of Dehgolan was almost differentiated (97.5 %) from other populations (Figure 2). Population classification based on standard morphometrics (14 variables) showed that DFA correctly classified 52.77% of the colonies table 5. Statistical analysis of four distances and eleven angles of front wings by DFA (standard morphometrics) showed that the honeybee population of Dehgolan was nearly separated (77.5 percent) from other areas (figure 2). Therefore, geometric morphometrics method could better discriminate honeybee populations than standard morphometrics.

Relations of geographical populations were evaluated by UPGMA (unweighted pair group method with arithmetic means) method. Results showed that topology of Dendrograms were almost similar. Dendrogram of four distances and eleven angles of front wings showed that two populations of Marivan and Sarvabad were similar and two populations of Dehgolan and Bijar separated from other populations (Figure 3). Dendrogram of 18 landmarks of Front wing showed that two populations of Sanandaj and Qorve were similar and Dehgolan populations completely separated from other populations (Figure 4). The both dendrograms based of standard and geometric morphometrics, honey bee populations of Bane, Qorve and Sanandaj classified each other.

DISCUSSIONS

With its wide range of geography, Iran harbors different *Apis mellifera meda* population adapted to various geographical regions extending from east to west and from south to north. Within this huge geography Rutther (1988) reported the presence of several Persian honey bee populations.

Wing shape likely driven by: (1) environmental pressures such as latitude (Alpatov, 1929), altitude (Verma *et al.* 1994; Hepburn *et al.* 2000) and climate (Hepburn *et al.* 2001; Radloff *et al.* 2005a, b; Tan *et al.* 2008); (2) sexual selection (Radloff *et al.* 2003a) and (3) abiotic factors such as temperature (Soose 1954) and season (Mattu & Verma 1984). Otherwise, wing angles were interrelated with phylogeny rather than phylogeography (Diniz-Filho *et al.*, 1999). Farshineh *et al.* (2007) compared *A. mellifera meda* populations of Iran (Orumieh, Tbriz, and Tehran) with populations in different zones of Turkey (Kiseher and Beypazari) and *A. mellifera carnica* of northern Turkey. They found that honeybee populations in Iran were smaller than honeybee populations in Turkey.

Morphometrics has been used widely for identifying honey bees populations. Morphometric identification techniques have improved considerably due to new computational techniques (Francoy *et al.*, 2008). The results of this study clearly showed that measurements of size and angle characters can be sufficient to identify or discriminate honey bee populations, but the new approach, geometric morphometrics, is simple and much more effective than standard

morphometrics in discrimination or identification of honey bee populations in Iran as well as in the world (Tofilski, 2008; Kandemir and Ozkan 2013).

The results presented here show that geometric morphometrics yielded better discrimination of honeybee populations (83.83%) than standard morphometrics (52.77). Tofilski (2008) demonstrated that geometric morphometrics (84.9%) yielded better discrimination of three honeybee subspecies (*A. m. mellifera*, *A. m. carnica* and *A. m. caucasica*) than standard morphometrics (83.8%). Kandemir and Ozkan (2013) compared standard morphometrics and geometric morphometrics ability to discriminate the honey bee populations distributed throughout Turkey. The results showed that geometric morphometrics was more successful (81.5%) than standard morphometrics (70.4%).

Venation was described either by 11 angles in standard morphometrics or by the coordinates of 18 landmarks (36 x, y coordinates) in geometric morphometrics. According to Bookstein (1991), the use of size-free coordinates instead of distances, rotations, or angles leads to more exhaustive descriptions of geometric forms in biology. While standard morphometrics is restricted to distance and ratios of distances, geometric morphometrics not only includes these measurements indirectly but also allows for wing shape analysis by using the landmark approach. The number of characters (36 x, y coordinates) used is thought to be more in geometric morphometrics and all the characters were size-free; thus, much better discrimination based on wing shape was not surprising.

CONCLUSIONS

Geometric morphometrics method is much simpler and easier compared to the standard morphometrics. Therefore, the discrimination ability of geometric morphometrics on the honey bee populations was better than standard morphometrics.

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APPENDICES

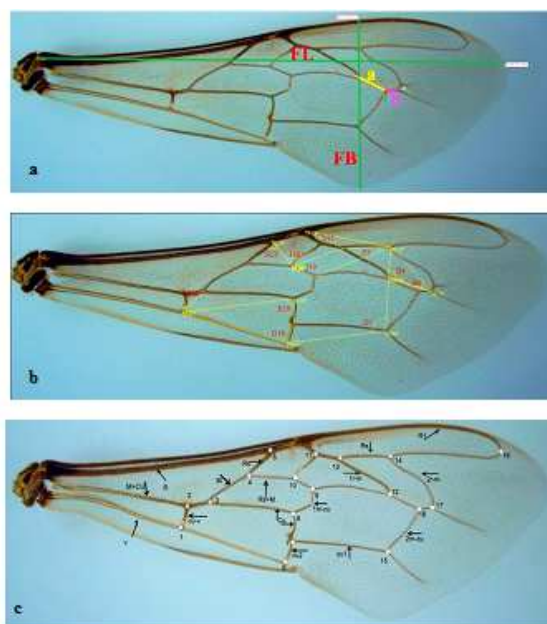


Figure 1 (a) Forewing Length (FL), Forewing Width (FB), Cubital Index ($\frac{FL}{FB}$);
(b) Angles of Fore Wing Venation; (c) Eighteen Landmarks
on Vein Junctions of Fore Wing of *Apis Mellifera*

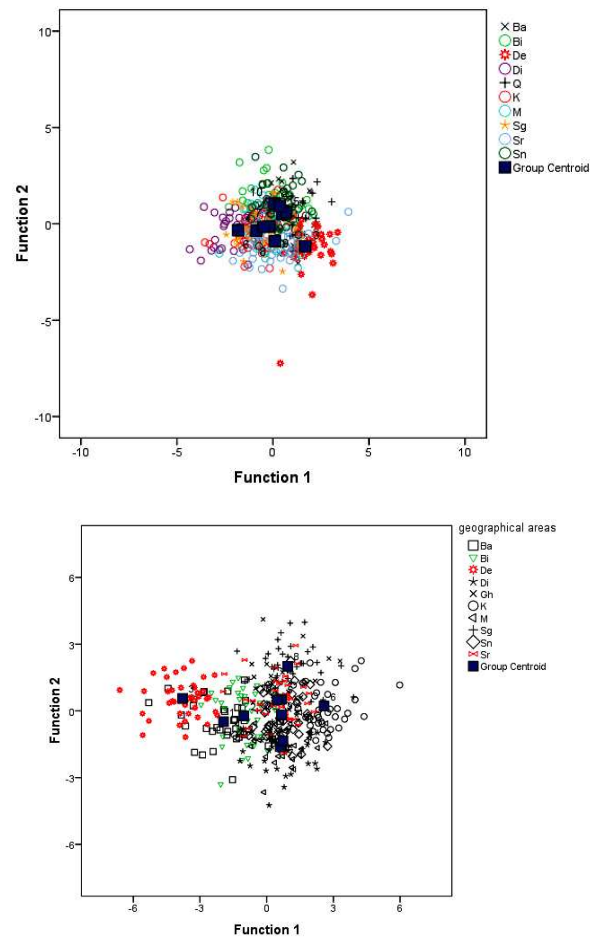


Figure 2: Discrimination of Honeybee Populations Based on Standard (a) and Geometric Morphometrics (b)

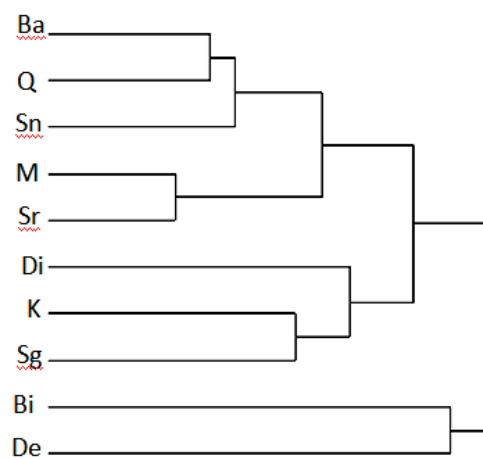


Figure 3: Dendrogram Resulting From a UPGMA Cluster Analysis of Samples From Geographical Populations Using Data from the Fore Wing by Standard Morphometrics

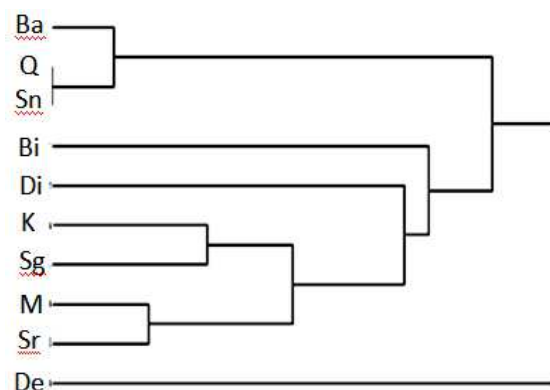


Figure 4: Dendrogram Resulting from a UPGMA Cluster Analysis of Samples from Geographical Populations Using Data from the Fore Wing by Geometric Morphometrics

Table 1: List and Code of Collecting Sites of *Apis mellifera* in Iran

Population	Code	Sample Number
Bane	Ba	40
Bijar	Bi	40
Dehgolan	De	40
Divandareh	Di	40
Curve	Q	40
Kamyaran	K	40
Marivan	M	40
Saghez	Sg	40
Sarvabad	Sr	40
Sanandaj	Sn	40

Table 2: Multivariate Analyses Based on Standard Morphometrics

	Value	F	Hypothesis df	Error df	Sig
Wilks lambda	.137	6.745	126	2863.702	.000

Table 3: Multivariate Analyses Based on Geometric Morphometrics

	Value	F	Hypothesis df	Error df	Sig
Wilks lambda	.007926	8.038	288	3104.6	.000

Table 4: Summary of the Colony Assignments with Respect to Regions Based on Standard Morphometrics Percent Classifications are in Parentheses; N Denotes the Number of Colonies

Groups	Ba	Bi	De	Di	Q	K	M	Sg	Sr	Sn
Ba	12(31.6)	-	-	-	-	-	-	-	-	-
Bi	-	19(47.5)	-	-	-	-	-	-	-	-
De	-	-	31(77.5)	-	-	-	-	-	-	-
Di	-	-	-	28(70)	-	-	-	-	-	-
Q	-	-	-	-	17(43.6)	-	-	-	-	-

K	-	-	-	-	-	25(62.5)	-	-	-	-
M	-	-	-	-	-	-	15(38.5)	-	-	-
Sq	-	-	-	-	-	-	-	20(50)	-	-
Sr	-	-	-	-	-	-	-	-	21(52.5)	-
Sn	-	-	-	-	-	-	-	-	-	21(52.5)

Table 5: Summary of the Colony Assignments with Respect to Regions Based on Geometric Morphometrics Percent Classifications are in Parentheses; N Denotes the Number of Colonies

Groups	Ba	Bi	De	Di	Q	K	M	Sg	Sr	Sn
Ba	35(92.1)	-	-	-	-	-	-	-	-	-
Bi	-	33(82.5)	-	-	-	-	-	-	-	-
De	-	-	39(97.5)	-	-	-	-	-	-	-
Di	-	-	-	36(90)	-	-	-	-	-	-
Q	-	-	-	-	25(64.1)	-	-	-	-	-
K	-	-	-	-	-	33(82.5)	-	-	-	-
M	-	-	-	-	-	-	35(89.7)	-	-	-
Sg	-	-	-	-	-	-	-	34(85)	-	-
Sr	-	-	-	-	-	-	-	-	29(72.5)	-
Sn	-	-	-	-	-	-	-	-	-	33(82.5)

Appendix I. Classification functions for discrimination of honeybee populations using mean of 40 forewings from one colony. The forewings were described by distances and angles between vein junctions.

Table 6

Subspecies	A4	B4	D7	E9	G18	J10	J16	K19	L13	N23	O26
<i>A. m. meda</i>	31.015	106.4	98.29	21.115	93.615	52.115	92.663	78.86705	14.3021	89.95199	34.82199

Appendix I. (Continued)

Table 7

Subspecies	FWL	FWW	CuA	CuB	CI
<i>A. m. meda</i>	9.0798	3.14	0.5978	0.25320	2.3611

